Guidelines for the Genotyping of Mice and Rats

Purpose

The proper identification of genetically engineered animals in a litter is critical to the efficient pursuit of research and in reducing the number of animals involved in a research project. Most often the genotype is determined by analysis of DNA extracted from tissues of young rodents. Analysis by the Polymerase Chain Reaction (PCR) requires the least amount of DNA. DNA for PCR analysis can be obtained from ear punches, hair or fecal samples, oral or rectal swabs (1-9). Depending on the requirements of the study, investigators are urged to consider these noninvasive alternatives. Larger amounts of DNA are required for Southern Blot determination of the genotype. Obtaining tissue from a mouse or rat for DNA analysis via tail biopsy is a safe, effective and humane procedure. When preformed properly it causes only minimal or transient pain and distress, and induces no more "physiological impact" (change in heart rate, body temperature, or activity level) than just restraining the animal for the procedure. (11) DNA prepared from tail biopsies is suitable for analysis by either Southern Blot or PCR.

Guidelines for Tail Biopsy

1. Procedures for tail biopsy for DNA analysis and/or genotyping must be described in an approved Animal Study Proposal (ASP).

2. Ideally, mice and rats should be **10-21** days old. At this age, the tail tissue is soft (vertebra are not yet calcified) and the yield of DNA is highest (8,10). In addition, prompt analysis of tail tissue allows the desired mice and rats to be identified prior to weaning which can facilitate more efficient use of cage space.

    a. **For mice and rats 10-21 days of age**: Because pain sensory development may be complete, and to further minimize any transient pain or distress, investigators are strongly encouraged to apply local anesthesia to the tail. Local anesthesia may be achieved by immersion of the tail in ice cold ethanol for 10 seconds, by an application of ethyl chloride spray or by the use of another suitable anesthetic as recommended by the attending veterinarian.
    b. **For mice and rats greater than 21 days of age**: The use of a local or general anesthetic is required prior to collection of tissue. If a general anesthetic is to be used, an appropriate agent should be recommended by the attending veterinarian.
    c. **For rats greater than 35 days of age**: The use of a general anesthetic is required.

3. Manually restrain the mouse or rat between thumb and forefinger. This is a convenient time to identify the animals using the appropriate method (i.e. ear punch, ear tag, transponder etc.).

4. With sterile scalpel, razor blade, or scissors cleanly excise the distal 2mm (maximum 5 mm) of the tail. If the proper procedures are followed, the yield of DNA from 5 mm of tail should exceed 50 micrograms, enough for multiple analyses. The yield of DNA does not proportionally increase as tail fragments larger than 5mm are used. If

small amounts of DNA are required, investigators should consider taking only 2 mm of tail. If the analysis of the DNA is to be performed by PCR, great care should be taken to remove all tissue from the scissors or scalpel after each animal. Disinfect the scalpel or scissors between animals. If a scalpel is used, also disinfect the work surface on which the tail is placed between animals.

5. The investigator must monitor the animals to assure hemostasis after the animals are returned to the cage. If needed, apply digital pressure, silver nitrate, or other means of hemostasis.

6. If additional DNA is needed for retesting alternatives to a second tail biopsy should be considered (11). Repeat tail biopsies require anesthesia and must be justified in the ASP. The use of post-procedural analgesia should be considered.

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